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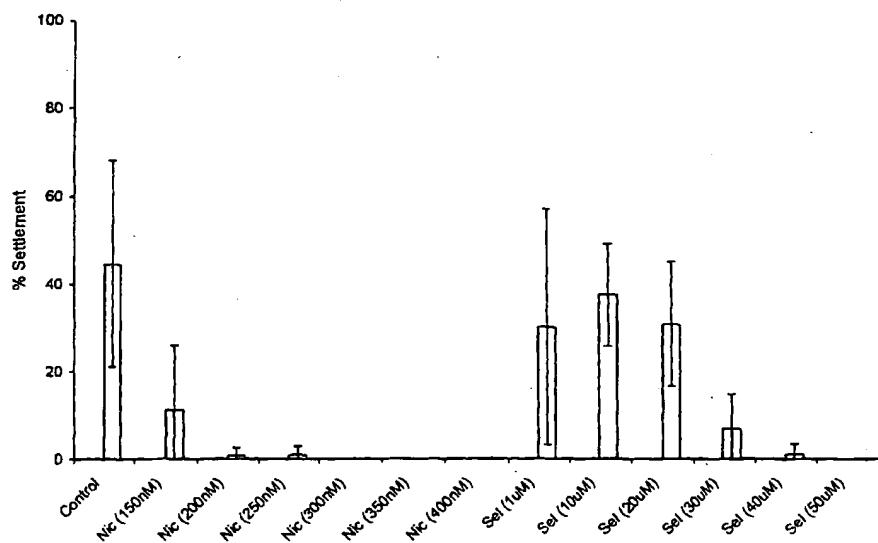
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**(54) Title:** A METHOD AND A SURFACE TREATMENT AGENT FOR PREVENTING BIOFOULING ON SURFACES UNDER WATER



**(57) Abstract:** An ecofriendly method of inhibiting biofouling on surfaces under water by using nicotine and selenium in the form of Se(0), or such a substance that can be converted into them. Both of the substances are necessary to oxygen dependent organisms and will be used after conversion, but are toxic in high doses. By adding the substances to paint or other surface treatment agent that marine surfaces are treated with, organisms that are trying to establish themselves on the surfaces will be exposed to so high doses, that reactions on which the settling is based are disturbed. When the substances leak out into the seas, they will act as environment protectors, as they promote the development of organisms.

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## A Method and a Surface Treatment Agent for Preventing Biofouling on Surfaces under Water

### TECHNICAL AREA

5 The present invention relates to a method of the kind stated in the preamble of claim 1. The invention also relates to a surface treatment agent of the kind stated in the preamble of claim 6.

### BACKGROUND OF THE INVENTION

10 Marine biofouling is a considerable problem to maritime activities. In most waters, an untreated ship hull will be fouled by plants and animals, which substantially increases the friction and, thereby, the fuel consumption. This can be most disturbing on propellers and propeller shafts. Also in connection with oil and gas production, there are problems with marine biofouling.

15 In the present context, the term "ship hull" does not relate merely to hulls for ships for commercial trade but also to hulls for small craft and boats for private use and to propellers and propeller shafts.

To prevent biofouling, the paints used today contain toxic metal compounds of tin or copper. This is no desirable solution, and several states have or intend to prohibit them.  
20 Therefore, research is intensified to provide alternatives that will spare the environment.

However, experience has shown that no non-toxic methods have been functional. In the present context, the term "functional" means that a surface treated to be antifouling will not be fouled by plants and animals. It is in the nature of the problem that only substances that are toxic to these biofouling organisms can prevent them from settling. Unless the reactions that form the basis for the binding to the surfaces will be superseded, the settling can not be prevented. In US-A-4,783,457 (Nathanson) there is disclosed how clonidine and similar substances may be used as pesticides on utility plants. They bind to an octopamine receptor, which is lacking in vertebrates, and this is understood as their being efficient especially against pests. The substances are reported to activate adenylyl cyclase (cyclic AMP is formed) and protein kinases and affect nerve functions by disturbing or blocking the signal transmission to effector cells. This means that they have an inactivating effect on energy generation, which in turn, eventually, results in the death of the organisms. The inactivation of the energy generation is disclosed more in detail in

1. Nyberg-Swenson, B. E., The selenium link: the missing link in our understanding of biochemical trigger reactions? Med. Hypothesis 1999, Vol.52, No.2, pages 125-131;

2. Nyberg-Swenson B. E., "Is molecular oxygen, O<sub>2</sub>, the reactive radical behind oxidations of (aut)oxidable agents to which the bases of DNA belong?", Med. Hypothesis 2002, Vol. 58, No.3, pages 203–212;
3. Nyberg-Swenson B. E., Are acetylcholine-induced acetylgroups driving fuel cells in the systems of transducin, τ and G proteins? Med. Hypothesis 2002, Vol. 58, No.5, pages 388–394.

WO 00/42851 (=SE 513 474 C2, Elwing, H. and Mårtensson, L.) suggests the use of medetomidine or a structural or biological analogue thereof, e.g. clonidine (catemines) to prevent fouling by crustaceans, especially acorn barnacles. Probably, the catemines take the place of another substance in the transport of electrons to oxygen, but without having the ability of said substance to transfer electrons, which prevents the energy formation. Catemines contain an imidazole ring and may bind to the same receptors as electron transferring substances having the same ring (adenine, guanine, (hypo)xanthine, etc.). Several types of receptors will activate adenylyl cyclase and protein kinases, and earlier tests with catemines have shown a toxic effect in tube building polychaetes and some kinds of fishes. If so, catemines are toxic to all oxygen dependent organisms.

WO 00/42851 states that "the cyprid larvae (which form acorn barnacles) unexpectedly react on catemines, which normally are active in vertebrates". As it is inevitable that the substances of boat bottom paints eventually will spread in the seas, and just like PCB, DDT and other substances will be enriched in organisms, catemines do not appear as a commendable alternative to other tested substances. Catemines are stable substances. If they would oxidise rapidly, they would lose their toxicity, but consequently also their functionality.

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#### DISCLOSURE OF THE INVENTION

The object of the present invention is to provide a method and a surface treatment agent, which are highly efficient against marine biofouling but nevertheless are environment friendly. This is achieved in that the method and the surface treatment agent, respectively, of the kind mentioned by way of introduction, are provided with the features stated in the characterizing clauses of claims 1 and 6, respectively.

By using, in accordance with the invention, substances, which are toxic in high doses but in lower doses will be converted into substances that are necessary for an organism, biofouling will be counteracted without the environment being negatively affected. Settling of crustaceans and other organisms on solid surfaces will be inhibited at the same time as the sea environment will be provided with substances that are wholesome for oxygen dependent

organisms. The invention comprises the use of at least one substance that is toxic on exposure in high doses but essential in low doses, as organisms can convert it into an important, necessary molecule for the generation of energy. The implications hereof are that a toxic substance, which falls within the scope of the present invention, will prevent biofouling but will not be toxic to organisms of the sea when leaking out. The problem of finding a surface treatment agent, which will be highly efficient against marine biofouling but in spite thereof will be environment friendly, is solved in a corresponding manner, in that the surface treatment agent for inhibiting biofouling and of the kind mentioned by way of introduction includes the features stated in the characterizing clause of claim 6.

In that the method and the surface treatment agent, of the kind mentioned by way of introduction, are provided with the features stated in the end portions of the characterizing clauses of claims 1 and 6, respectively, a method and a surface treatment agent are achieved, which are highly efficient against marine biofouling but nevertheless are environment friendly.

Suitably, the method and the surface treatment agent, of the kind mentioned by way of introduction, are provided with the features stated in the characterizing clauses of claims 2–5 and 7–9, respectively.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a diagrammatical representation of the lethal dose and the settlement-inhibiting effect of two compounds in accordance with the invention, nicotine and sodium selenite, on cyprid larvae.

Figure 2 are diagrammatical representations showing the effective concentration (when no cyprids were settled) of nicotine and of sodium selenite.

Figure 3 is a diagrammatical representation of the synergistic effect obtained when using nicotine and sodium selenite simultaneously for preventing the settling of cyprid larvae.

#### DESCRIPTION OF THE PREFERRED EMBODIMENTS

The settling of crustaceans and other organisms on boat keels is a serious problem to maritime activities in many countries. Up to now, only toxic substances in paints or other surface treatment agents have proved to be able to inhibit biofouling. But when leaking out, these substances are just as toxic to sea organisms. They do not decompose, but accumulate in the seas. Consequently, it is very important to find alternatives that will spare the environment.

The invention includes the use of the substances referred to in the characterizing clauses of claims 1-2 and 6-7, respectively. In the search among substances that are toxic on exposure in high doses but essential in low doses as organisms then can convert them into important, necessary molecules for the generation of energy, nicotine and selenium in atomic form, Se(0), stand out as very promising. Dilute solutions of nicotine earlier have been used as pesticides, but they were banned due to their high toxicity. As they often were handled by persons, who were not sufficiently aware of how dangerous the substance is, (fatal) accidents occurred among both children and adults.

But nicotine amide, after formation of nicotine amide dinucleotide ( $\text{NAD}^+$ ), is an extremely important substance in connection with the transport of hydrogen (electrons and protons) to oxygen, which is the precondition for the energy formation of the cells and, thereby, the existence of life. Nicotine will be converted into nicotine amide by organisms and will bind to the same receptors as acetylcholine (3. Nyberg-Swenson B. E., Are acetylcholine-induced ..., *loco cit.*). The reactions of these substances control the activity between muscle and nerve cells or between nerve cells. At a too high or too fast supply of nicotine, which may cause the death of an organism, the nicotine probably will not have time enough for the conversion into nicotine amide, but will bind directly to the receptors. But nicotine is lacking the important ability of transferring charges. The resulting inactivity, the lack of energy, is devastating to an organism.  $\text{NAD}^+$  can also promote fermentative processes that admittedly produce energy, but not in an amount sufficient to sustain life in a highly advanced organism.

Fermentative processes can also be promoted when the transport of electrons to oxygen is disrupted or insufficient. An example hereof is the formation of lactic acid in connection with a heavy physical strain. But simultaneously the amount of NADH will increase. Hydrogen and an electron therein will be used subsequently, when the normal transport of hydrogen and electrons to oxygen has begun running properly again. An important link among many others in the chain of hydrogen transporters is the selenium link. It contains two selenium particles of different valencies (-II and 0), of which the first selenium particle is substituted for sulphur in a protein bound cysteine (1. Nyberg-Swenson, B. E., The selenium link, ... *loco cit.*). Until 1952, when selenium was found to be an essential substance for animals and could prevent muscular dystrophy, the substance was regarded exclusively as a poison. If Se(0) or a selenium salt (selenite, selenate, etc.) in high doses is supplied to an organism, this results in an excretion of Se(0) together with the other Se form (1. Nyberg-Swenson, B. E., The selenium link, ... *loco cit.*). The content of Se(-II)

may get so low that the organism will be harmed or even die. Because thereof, Se(0) and selenium salts are classified as strong poisons.

Since both nicotine amide and the selenium link are necessary substances in the transport of hydrogen (or electrons and protons) in the same chain, too high a supply of nicotine and Se(0) or a selenium salt will result in synergistic toxic effects.

The development of many organisms occurs to a great extent to solid surfaces. The organisms use substances in the settling material, which are necessary for their development. Without chemical reactions and bindings with these substances, the organisms would neither be able to settle or to live on the surfaces. That has been found for bacteria that develop in connection with minerals (PEDERSEN, K., "Diversity and activity of microorganisms in deep igneous rock aquifers of the Fennoscandian shield", In: Subsurface Microgeobiology and Biogeochemistry. Edited by Fredrickson J. K. and Fletcher M., 2001, ISBN 0-471-31577-X), and higher organisms, such as acorn barnacles, for example, most likely are no exception. Consequently, for its development, no organism can use a substrate that involves exposure to high doses of poisons, such as nicotine and/or Se(0), for example. In practice, such a substrate is achieved by using a surface treatment agent according to claim 7, and suitably having anyone of the features claimed in the characterizing clauses of claims 10 and 11.

With at least one of nicotine and Se(0) or substances that can be converted into them (nornicotine, myoamine, anabasin, selenite, selenate, etc.) in surface treatment agents (e.g. boat bottom paints) that are to protect against biofouling of surfaces in a marine environment, biofouling will be inhibited thanks to the toxic effect of the substances. That an organism could avoid the substances if they are included in surface treatment agents (paints) on treated (painted) surfaces, e.g. boat keels, on which they try to settle, is not to be thought of, since the substances can bind to receptors of the organism. That is the reason why a nicotine stain on an article of clothing may be sufficient to harm the wearer.

With the biofouling inhibiting agents incorporated in a polymer that is included in the paints or other surface treatment agents for marine surfaces, their leaking out into the surroundings too rapidly will be prevented. But leakage is inevitable. However, due to the ensuing dilution effect, the content of the substances in the sea will be very low. With the very low dose levels in question, nicotine and selenium will have only positive effects. There will be no risk of any organism in the sea being exposed to toxic doses in any other way than by contact with the treated surfaces, on which the organisms are to be avoided. Instead, the substances will serve as environment protectors, as they will promote the development of organisms.

Paints that are suitable for the incorporation of the biofouling inhibiting agents may be quite conventional standard paints, e.g. acrylate based paints, wherein the polymer is polyacrylate.

In comparison to all existing or proposed substances, nicotine and Se(0) are not only non-toxic but are also valuable substances for all oxygen dependent organisms in the concentration that they may occur in the seas in connection with a utilization for inhibiting biofouling in marine environments.

Selenium is of extreme importance and is very delicate, since only a very small amount is required for a good function. Many of the selenium sources of Earth presently are inactivated by the strong bindings of the substance to several metals (Hg, Cd, Pb, etc.), DDT, PCB, freons, and other halogen organic compounds – the only cause of the toxicity of the substances (2. Nyberg-Swenson B. E., "Is molecular oxygen...", *loco cit.*). In Finland, selenite has been successfully added to soil over large areas. Most likely, seas would benefit from the same measure, which can be carried out via boat bottom paints. The positive environmental effect will be two-fold.

A further development of the innovation is facilitated by the knowledge of how the above mentioned substances react with organisms, something that is unknown with other used substances.

Nicotine and Se(0) are stable substances, well suited for incorporation in polymeric paint intended for the coating of marine structures.

As a complement, the substances in question may be applied exteriorly or interiorly of tubes and other marine structures by (high-pressure) spraying, even if it is inevitable that spraying liquid will be spread in the surroundings. This will cause no inconvenience from an environmental point of view, provided that protective measures against exposure hazards for the individuals involved are taken.

The invention will now be illustrated further by means of the following examples, which are in no way intended to restrict the scope of protection of the invention.

#### Example 1

This example illustrates the lethal dose and the settlement-inhibiting effect of two compounds in accordance with the invention, nicotine and sodium selenite (below referred to as "selenite"), on cyprid larvae. Cyprid larvae from *Balanus improvisus* were transferred to Petri dishes of polystyrene containing seawater from the Swedish west coast and nicotine or sodium selenite in various concentrations (5 mM, 500 µM, 50 µM, 5 µM, 0.5 µM and 50 nM). In addition, for control purposes, cyprid larvae were transferred to a Petri dish

containing seawater only.  $20 \pm 5$  cyprid larvae were used in each Petri dish and 5 Petri dishes contained the same concentration. After 6 days the cyprids were counted and classified as either dead or settled (established) on the surface of the Petri dish. The rest were swimming, i.e. living but not established.

5 The result is set forth in the diagram shown in Figure 1 which illustrates the first settlement trial to establish coarse effect concentration and lethal dose of nicotine and selenit. The results are expressed in percentage of the total number of organisms in each dish ( $20 \pm 5$ ) and the mean value and deviation is calculated for the 5 dishes. The experiment shows that the number of dead cyprids is 100 % in 5 mM nicotine and almost 100 % in 500  $\mu\text{M}$  (0,5 mM) selenite. From this experiment appears that the number of established larvae decreased upon increased concentration of nicotine or selenite. To further investigate the effective concentration (when no cyprids were settled), the experiment described in Example 2 was performed.

10

15 Example 2

The procedure in Example 1 was repeated with the exceptions that the concentrations of nicotine were: 400 nM, 350 nM, 300 nM, 250 nM, 200 nM, 150 nM and of sodium selenite: 1  $\mu\text{M}$ , 10  $\mu\text{M}$ , 20  $\mu\text{M}$ , 30  $\mu\text{M}$ , 40  $\mu\text{M}$ , 50  $\mu\text{M}$ .

20 The results are set forth in the diagram shown in Figure 2 which illustrates the second settlement trial to establish the exact effect concentration of nicotine and selenit. The results are expressed in percentage of the total number of organisms in each dish ( $20 \pm 5$ ) and the mean value and deviation is calculated for the 5 dishes. The experiment shows that the effective concentration (when no cyprids were settled) of nicotine is 300 nM (0.3  $\mu\text{M}$ ) and of selenite 50  $\mu\text{M}$ .

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Example 3

This example corresponds to Example 1 and 2 with the exception that the Petri dishes contained both nicotine and selenite according to the diagram shown in Figure 3. It illustrates the third settlement trial to examine synergistic effects of nicotine and selenit.

30

The results are expressed in percentage of the total number of organisms in each dish ( $20 \pm 5$ ) and the mean value and deviation is calculated for the 5 dishes. In a sea water solution of 50 nM nicotine and 10  $\mu\text{M}$  selenite,  $60 \pm 30$  % cyprids were settled and in 150 nM nicotine and 10  $\mu\text{M}$  selenite,  $20 \pm 10$  % cyprids were settled. In 50 nM nicotine and 30  $\mu\text{M}$  selenite,  $10 \pm 10$  % cyprids were settled and the value was further reduced to  $2 \pm 5$  % in 150 nM nicotine and 30  $\mu\text{M}$  selenite.

The experiment shows that the number of settled cyprids diminished by a combination of nicotine and selenite compared to only one substance and was further reduced by an elevated level of nicotine and/or selenite. The results demonstrate a synergistic effect between nicotine and selenite, especially in moderate concentrations (i.e. 50-150 nM  
5 nicotine and 20 µM selenite), regarding their ability of preventing cyprid larvae from settling on the utilised Petri dishes compared to the percentage of settled cyprids in the control dishes.

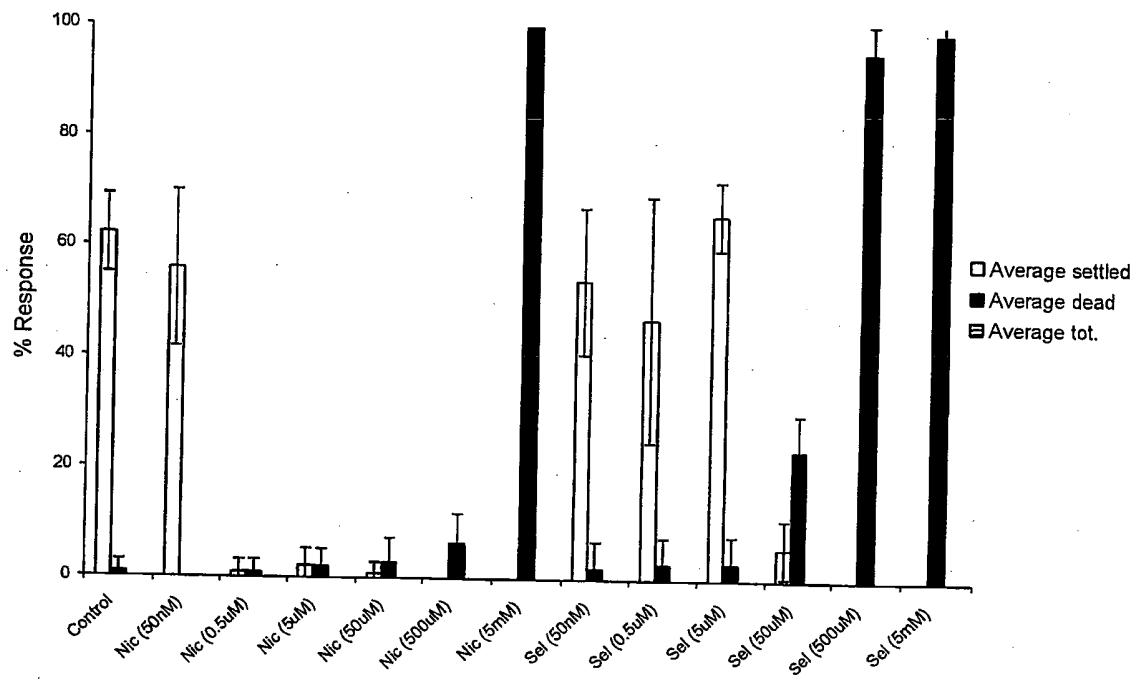
Only routine experimentation by the skilled art worker, and no inventive activity, is required to establish the optimum concentrations of nicotine and selenite in coating mixtures  
10 for marine structures to completely prevent cyprid larvae from settling.

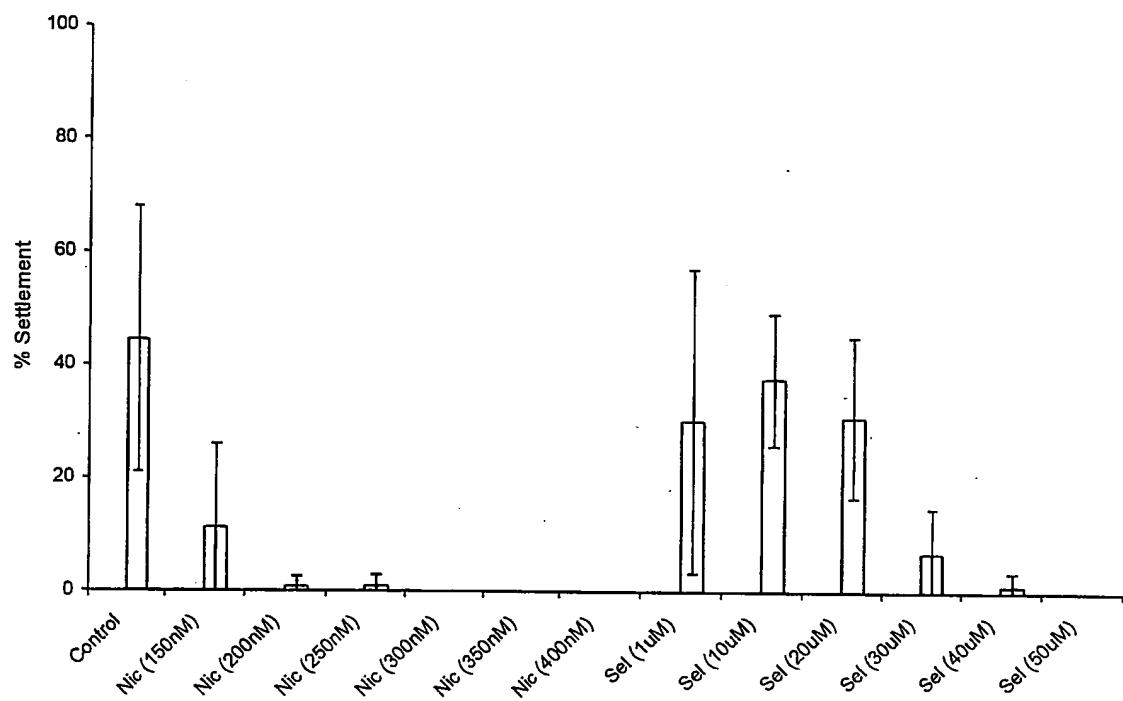
## C L A I M S

1. A method of inhibiting marine organisms, such as acorn barnacles or mussels, for example, from settling on a solid surface by applying on said surface at least one active substance that inhibits biofouling, characterized by selecting as said at least one active substance a substance that is toxic in high doses but will convert into a substance that is necessary for said organism and is functional in low doses, and – to inhibit biofouling by an oxygen dependent organism – selecting as said at least one active substance a substance that on one hand holds back transport of hydrogen (electrons and protons) to oxygen (which is a prerequisite of the formation of energy in nerve and muscle cells and, thereby, their activity, which is a prerequisite of a settling), and on the other hand will be used after conversion by the oxygen dependent organism.  
5
2. A method as claimed in claim 1, characterized by selecting as said at least one hydrogen transport holding back substance at least one of nicotine and selenium in atomic form, Se(0), or substances that can be converted into them (nornicotine or other nicotine derivatives, myoamine, anabasin, selenite, selenate, etc.).  
10
3. A method as claimed in any one of claims 1–2, wherein said solid surface is a marine structure.  
15
4. A method as claimed in claim 3, wherein said solid surface is a ship hull.
5. A method as claimed in claim 3, wherein said solid surface is a pipe conduit in sea water.  
20
6. A surface treatment agent for application on a solid surface for inhibiting the settling of marine organisms, such as crustaceans (acorn barnacles) or mussels, for example, on said surface, said surface treatment agent including a mixture of at least one active substance that inhibits biofouling and a carrier for the active substance, characterized in that as said at least one active substance a substance is selected that is toxic in high doses but will convert into a substance that is necessary for said organism and is functional in low doses, and that it on one hand holds back transport of hydrogen (electrons and protons) to oxygen (which is a prerequisite of the formation of energy in nerve and  
25

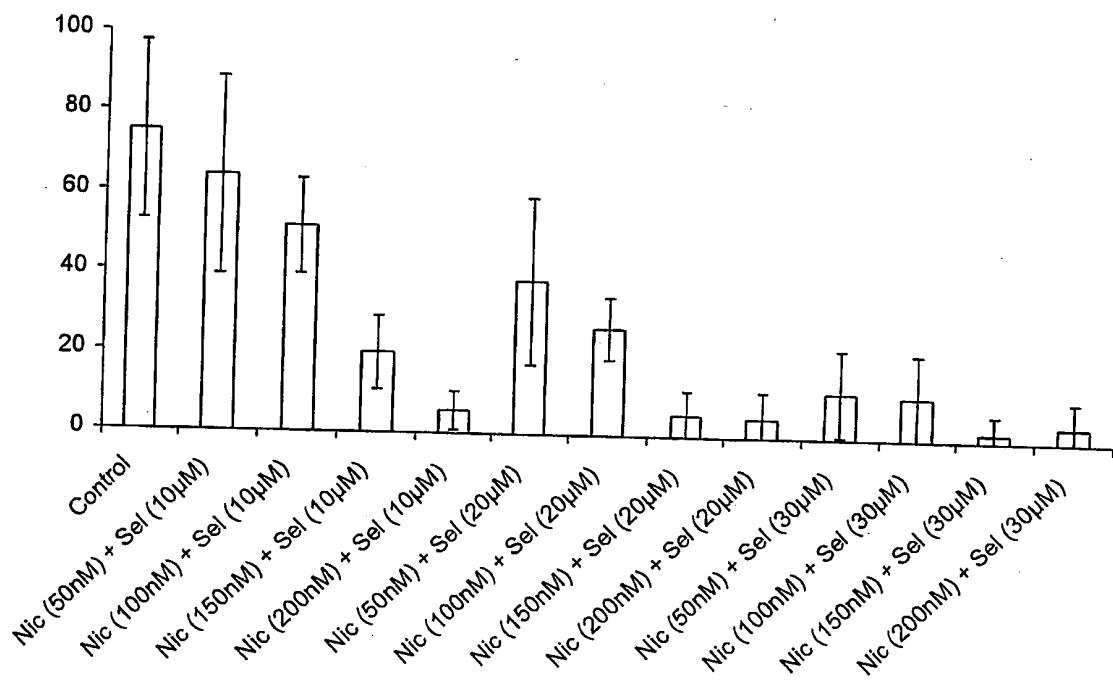
muscle cells and, thereby, their activity, which is a prerequisite of a settling), and on the other hand will be used after conversion by the oxygen dependent organism.

7. A surface treatment agent as claimed in claim 6, characterized in that said as said at  
5 least one hydrogen transport holding back substance at least one of nicotine and selenium in atomic form, Se(0), is selected, or substances that can be converted into them (nornicotine or other nicotine derivatives, myoamine, anabasin, selenite, selenate, etc.).
- 10 8. A surface treatment agent as claimed in any one of claims 6–7, characterized in that said carrier is a polymer that prevents the active substance from leaking out fast.
- 15 9. A surface treatment agent as claimed in claim 8, characterized in that said polymer is a paint for painting surfaces that will be exposed to marine biofouling, e.g. ship hulls and pipe conduits in sea water.

**Fig. 1**



**Fig. 2**



5

**Fig. 3**

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 2003/002066

## A. CLASSIFICATION OF SUBJECT MATTER

**IPC7: C09D 5/16**

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

**IPC7: C09D**

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0954966 A1 (ROHM AND HAAS COMPANY), 10 November 1999 (10.11.1999), abstract, (0023)  --	1-9
A	GB 982587 A (EMANUELE MOR), 10 February 1965 (10.02.1965), claim 1  --	1-9
A	US 6365066 B1 (WOLFGANG PODSZUN), 2 April 2002 (02.04.2002), column 5, line 1, abstract  -----	1-9

 Further documents are listed in the continuation of Box C. See patent family annex.

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## INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

27/02/2004

PCT/SE 2003/002066

EP	0954966	A1	10/11/1999	AU	758109	B	13/03/2003
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